STANDARD OPERATING PROCEDURE
BLOOD ALCOHOL ANALYSIS (METHOD 2)
(Revised 07/01/07)

Principle:

Headspace chromatography is based on Henry's Law which states, for a dilute solution, the solubility of a gas in a liquid expressed as a mole fraction depends upon the pressure of the gas. There is a fixed ratio between the mole fraction of the gas in the air and the mole fraction in the liquid. This ratio remains constant for a given temperature.

Specimens:

Optimum sample volume: 3 mL or greater. Samples which contain less than 3 mL may be analyzed.

Acceptable specimens are whole blood, urine, biological fluids or other alcoholic solutions. Other specimens may be analyzed with the approval of the laboratory director or designee.

Blood: The preferred sample is blood that is submitted in the ND Crime Laboratory BAC Kit. These kits have sterile Vacutainer tubes containing sodium fluoride and potassium oxalate.

Urine: The preferred sample is urine that is submitted in the ND Crime Laboratory Urine Kit. These kits have urine bottles containing sodium fluoride.

Refrigerators may be used for specimen storage.

Equipment:

1. Gas Chromatograph (GC)
   a. Varian 3400Cx
      1. FID detector or equivalent for volatiles
      2. Column temperature: 40°C
      3. Restek Rtx-BAC1, Restek Rtx-BAC2 or equivalent column
      4. Gas flows:
         a. Hydrogen Carrier: 14 mL/min
         b. Hydrogen FID: 17 mL/min
         c. Nitrogen FID: 28 mL/min
         d. Air FID: 300 mL/min
         e. Split: 15 mL/min
         f. Septum Purge: 5 mL/min
      5. Injector temperature: 200°C
      6. Detector temperature: 200°C
   b. PerkinElmer Clarus500
      1. FID detector or equivalent for volatiles
      2. Column temperature: 45 °C
      3. Restek Rtx-BAC1, Restek Rtx-BAC2 or equivalent column
      4. Gas flows:
         a. Hydrogen Carrier: 25 psi (~16 mL/min)

b. Hydrogen FID: 45 mL/min
c. Air FID: 450 mL/min
d. Split: Off
e. Septum Purge: ~ 5 mL/min
5. Injector temperature: 150°C
6. Detector temperature: 220°C

2. Headspace Autosampler
a. CombiPal (CTC):
1. Cycle: HS-INJ
2. Syringe: 2.5 mL-HS
3. Sample Volume: 1000 µL
4. Incubation Temp: 60.0 °C
5. Incubation Time: 0:12:00
6. Agitation Speed: 250 rpm
7. Agitation Time On: 5 s Agitation Time Off: 55 s
8. Syringe Temp: 75 °C
9. Syringe Fill Speed: 100 µL/s
10. Inject To: GC Inj1
11. Inject Speed: 250 µL/s
12. Pre Injection Delay: 500 ms Post Inj. Delay: 500 ms
13. Syringe Flushing: 0:02:30
14. GC – Runtime: 0:03:30

b. PE TurboMatrix 110
1. Temp Screen
a. Carrier: 35 psi
b. Needle: 110 °C
c. Transfer Line: 120 °C
d. Thermostat Oven: 70 °C
2. Timing Screen
a. Pressurize: 1.5 min
b. Inject: 0.2 min
c. Withdraw: 3.0 min
d. Thermostat: 12.0 min
e. GC Cycle: 4.3 min
f. PII: 6.00 min (calculated by autosampler)
3. Option Screen
a. Operating Mode: Constant
b. Inject Mode: Time
4. PPC Screen Not installed
5. Needle Purge ~ 15 mL/min (manual setting)

3. Note: Temperatures, pressures and other parameters for Equipment 1 and 2 are suggested operating conditions and may need to be altered to obtain optimum chromatographic results.
4. Atlas Chromatography Data System, chromatography software or integrator
5. Vials, caps, septa and crimper
6. Other laboratory supplies:

a. Pipettes (SMI and/or equivalent)
b. Automatic delivery pipette or Repipet
c. Weighing bottles and lids
d. Volumetric flasks and stoppers (various sizes)
e. Analytical balance
f. Polyethylene bottles (500 mL)
g. Storage vials with caps
h. Beakers (various sizes)
i. Pipettes
j. Note: As determined by analysts; appropriate lab supplies, equipment or glassware may be substituted for analytical procedure.

Reagents:

n-Propanol, $CH_3CH_2CH_2OH$, analytical grade. **Flammable, may be harmful if swallowed, inhaled or absorbed through the skin.**

Ethanol, CH_3CH_2OH, 200 proof, USP grade. **Flammable, may be harmful if swallowed, inhaled or absorbed through the skin.**

Sodium fluoride, NaF, analytical grade. **May be fatal if inhaled or swallowed.**

Sodium hydrosulfite, $Na_2S_2O_4$, analytical grade. **Flammable. May ignite with moisture and air. Harmful if swallowed. Causes irritation.**

Ammonium sulfate, $(NH_4)_2SO_4$, analytical grade.

Ethanol Calibrators: Made with 200 proof USP grade ethanol and reverse osmosis distilled water. Store in refrigerator. Expiration date is 2 months from date of preparation.

Diluent: Made with analytical grade ammonium sulfate, analytical grade sodium hydrosulfite and reverse osmosis distilled water. Store at room temperature. Expiration date is 6 months from date of preparation.

Internal Standard: Made with analytical grade n-propanol and diluent. Store at room temperature. Expiration date is 6 months from date of preparation.

Blank Blood: **Use Universal Precautions when handling biohazardous material.** Prepared with whole blood or packed Red Blood Cells (RBCs) from United Blood Services of Bismarck or equivalent vendor and adding analytical grade sodium fluoride. Prepare at a concentration of 10 mg/mL (10 mg sodium fluoride per 1 mL blood or RBCs). Expiration date is 4 months from date of preparation. Store in refrigerator.

Aqueous Commercial Controls: Target concentration of 0.10 g% ethanol by weight. Concentration ranges of controls may be used between 0.02 to 0.50 g% ethanol by weight.

Expiration date is determined by manufacturer. Store at room temperature or in refrigerator.

Commercial ethanol standards may be purchased. Concentration range of 0.002 g% to 1.000 g%. Expiration date is determined by manufacturer. Store at either room temperature or refrigerate until opened. Once opened, store in refrigerator.

Preparing Standard Ethyl Alcohol Solutions: (See Table I)

1. Use calibrated pipettes (or equivalent).
2. Use pure anhydrous ethyl alcohol.
3. Fill the appropriate size volumetric flask to about 4/5 full with distilled water.
4. Place approximately 10-15 mL of distilled water into a weighing vessel. If required, fit with lid. Place on analytical balance and tare.
5. Deliver the pure anhydrous alcohol to the weighing vessel and note the weight in grams to 4 decimal places.
6. Quantitatively transfer the contents of the weighing vessel into the appropriate size volumetric flask, rinsing the weighing vessel several times with distilled water.
7. Fill the volumetric flask to the mark with distilled water and mix contents thoroughly.
8. Transfer into 500 mL polyethylene (or equivalent) bottles and store in refrigerator.
9. Check new standards against the previous standards for accuracy. Analyze 4 samples of each by GC/HS. Standards < 0.10 g% must be within ±0.005 g% of weighed value, while standards > 0.10 g% must be within 5% of weighed value.
10. Commercially-prepared ethanol standards may also be used for the preparation of the calibration curve.

TABLE I
(These solutions will be used in preparing the calibration curve)

Target Alcohol Concentration (g%)	Volumetric Flask Size	Standard Solution Concentration
0.015	1 L	0.015 g% ± 0.005 g%
0.050	0.5 L	0.050 g% ± 0.005 g%
0.150	0.5 L	0.150 g% ± 5%
0.350	0.5 L	0.350 g% ± 5%
0.550	0.5 L	0.550 g% ± 5%

Preparation of Diluent and Internal Standard Solutions:

1. The Diluent Solution is prepared by dissolving 132 grams of ammonium sulfate and 17.4 grams of sodium hydrosulfite per liter of reverse osmosis distilled (ROD) water.
2. The Internal Standard (IS) solution is prepared by diluting a weighed volume (~250 µL) of n-propanol per liter of diluent solution to obtain a concentration within the range of 0.018 g% to 0.022 g%.

Preparation of Volatiles Solution:

1. The "volatiles" solution is a dilution of 25-50 µL each of methanol, acetone, ethanol, iso-propanol and n-propanol into a 100 mL volumetric flask.
2. The flask is approximately half filled with distilled water before the addition of the various volatiles and then filled to the mark with distilled water.
3. Invert several times to mix.
4. This solution is for qualitative use only.
5. Store in refrigerator.

Preparation of Standards, Controls, Case Samples, Blank and Zero for Analysis:

1. Table II summarizes the preparation of each required item for analysis. Table III shows the procedure for preparing the calibrators.
2. Each standard ethyl alcohol solution is prepared in singlet. Blank, zero and volatiles are prepared in singlet.
3. Commercial controls are prepared as needed. Case samples are prepared in duplicate. Samples and controls may be analyzed more than once.
4. Once all components are placed in a labeled vial, it is capped and crimped.
5. The number of controls should not be less than 25% of the case samples being tested.

TABLE II
Preparation for Analysis

	Volume Used	Amount of Blood Added	Amount of ROD H_2O Added	Amount of Diluent Added	Amount of IS Solution Added
Standards	100 µL	100 µL	---	---	2 mL
Commercial Controls	100 µL	100 µL	---	---	2 mL
Blank	---	100 µL	100 µL	2 mL	---
Zero	---	100 µL	100 µL	---	2 mL
Volatiles	100 µL	100 µL	---	---	2 mL
Sample – Blood	---	100 µL	100 µL	---	2 mL
Sample – Urine or Aqueous Sample	100 µL	---	100 µL	---	2 mL

Table III
Standard Curve Preparation

Standard Target Concentration	Amount Standard Added	ROD H_2O	Blank Blood	Internal Standard Solution
0.015 g%	100 µL	---	100 µL	2 mL
0.050 g%	100 µL	---	100 µL	2 mL
0.150 g%	100 µL	---	100 µL	2 mL
0.350 g%	100 µL	---	100 µL	2 mL
0.550 g%	100 µL	---	100 µL	2 mL

Sample Analysis:

1. An autosampler worksheet is prepared indicating the position of each vial in the carousel or tray of the autosampler. The worksheet will be identified by gas chromatograph and date.
2. The proper sequence for beginning an alcohol analysis would be to run the 5 ethyl alcohol standards, blank, zero and volatiles solutions; followed thereafter by a constant pattern of a control, case sample (in duplicate) and ending with a control.
3. The standard curve should be analyzed by linear regression analysis. The correlation coefficient of the line will be calculated. If the correlation coefficient is not greater than or equal to 0.999, then the standard curve should be rerun.
4. As long as the concentrations of controls continue to be in the acceptable range, there is no need to rerun the standard curve.
5. Upon completion of the analysis, the position and identity of the vials should be compared to the worksheet to verify the injection sequence prior to the removal of the vials from the autosampler carousel/tray. If the vial positions do not match, an explanation should be noted on the autosampler worksheet.
6. If the concentration of a sample is greater than the highest standard concentration, the sample may be diluted quantitatively with ROD water and then reanalyzed. The tested alcohol concentration will then be calculated according to the amount of the dilution. The final value of the alcohol concentration should be noted on Form 101 and the analytical report.

Acceptable Criteria:

1. The concentration coefficient, as determined via linear regression analysis, of the five standards must be ≥ 0.999.
2. The reported concentration of all controls > 0.10 g% must be within 5% of the expected value. Controls < 0.10 g% should be within 0.005 g% of the expected value. If a control falls out of range, the sample on either side of the control should be repeated along with a control. However, if any control falls out of acceptable range, the case sample prior to and immediately following that control must be reanalyzed. All copies of Form 101 will be retained in the sample file.

3. In the analysis of a case sample, the deviation between the average and either one of the two concentration values should not be greater than 3%. If the difference is greater than 3%, then the case sample must be re-tested.

Sample Reporting:

1. The case results should be generated as Form 101.
2. The Analytical Report (Form 107 or 107-U) should be completed by the analyst.
3. If the size of the sample submitted is less than what is necessary to perform the analysis in duplicate, the Analytical Report (Form 107 or 107-U) should reflect that the quantity is not sufficient for analysis.
4. A certified copy of the Submission Form (Form 104 or 104-U) and Analytical Report (Form 107 or 107-U) should be prepared and sent to the submitting agency.
5. The result should be recorded on the Case Jacket Review, if applicable.
6. A peer review of the analysis will be performed before the reporting of results.
7. Chromatograms and data will be printed out on an as-needed basis.

Headspace Analysis Files and Chromatograms:

1. A headspace file will be generated to include:
   a. Standards, blank, zero and volatiles chromatograms.
   b. Alcohol analysis worksheets.
   c. Computer generated spreadsheet or chromatography software information, QC summary and sample summary.
2. At a minimum, the file will be labeled as headspace analysis, date and analyst's initials.
3. The headspace files will be filed chronologically.
4. Chromatograms and raw data are stored electronically on a computer server.
5. Data will be analyzed and reported by either computer spreadsheet or chromatography software.

Sample Storage:

1. After reporting the results to the submitting agency, the sample should be placed in an appropriate storage container. The case sample numbers will be marked on a Blood Tube Storage Worksheet and placed on the outside of the container.
2. The samples should be placed in an appropriate long-term storage unit.

Manufacturers and vendors for chemicals, reagents and supplies include, but are not limited to:

Aaper Alcohol
Thermo Fisher
Varian, Inc.
Nerl Diagnostics
Restek Corporation
PerkinElmer LAS